Requirement of the activity of hepatocyte growth factor activator inhibitor type 1 for the extracellular appearance of a transmembrane serine protease matriptase in monkey kidney COS-1 cells

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Abstract Hepatocyte growth factor activator inhibitor type I (HAI-1) is a membrane-bound, serine protease inhibitor with two protease-inhibitory domains (Kunitz domain I and II). HAI-1 is known as a physiological inhibitor of a membrane-bound serine protease, matriptase. Paradoxically, however, HAI-1 has been found to be required for the extracellular appearance of the protease in an expression system using a monkey kidney COS-1 cell line. In the present study, we show using COS-1 cells that co-expression of recombinant variants of HAI-1 with the inhibition activity toward matriptase, including a variant consisting only of Kunitz domain I (the domain responsible for inhibition of matriptase), allowed for the appearance of this protease in the conditioned medium, whereas that of the variants without the activity did not. These findings suggest that the inhibition activity toward matriptase is critical for the extracellular appearance of protease in COS-1 cells.

Keywords Extracellular occurrence of matriptase · Hepatocyte growth factor activator inhibitor type 1 · Intracellular environments · Kunitz domain · Matriptase-inhibitory activity

Abbreviations

HAI-1	Hepatocyte growth factor activator inhibitor type I
HGF	Hepatocyte growth factor
HRP	Horseradish peroxidase
LDLRA domain	Low-density lipoprotein receptor A domain
MMPs	Matrix metalloproteinases

Introduction

Hepatocyte growth factor (HGF) activator inhibitor type 1 (HAI-1) is a Kunitz-type serine protease inhibitor, which is strongly expressed in epithelial elements of organs (Shimomura et al. 1997; Kataoka et al. 1999). HAI-1 is expressed first as a membrane-anchored form with a molecular mass of 66 kDa (refer to Fig. 1) (Shimomura et al. 1999). The extracellular domain, which contains two protease-inhibiting Kunitz domains, is shed from cell-surface membranes possibly via cleavage with certain matrix metalloproteinases (MMPs) (Shimomura et al. 1999). To date, HAI-1 has been found to inhibit serine

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Fig. 2 Structure and expression in COS-1 cells of WT-matriptase. **a** The structure of non-activated (*upper*) and activated forms (*lower*) of WT-matriptase. The amino acid numbering starts from the putative N-terminus of the protein. The N- and C-termini are indicated by NH₂ and COOH, respectively, in the non-activated WT-matriptase. The predicted disulfide linkage between two cysteine residues corresponding to Cys604 and Cys731 in matriptase are shown as SS. In the illustration of non-activated form, the site of antibody production (Spr992) is indicated by *underlining* in the amino acid sequence in single-letter code. The amino acid sequence around the matriptase activation cleavage site is also indicated with amino acid numbering at the N-terminal Val residue of the catalytic domain (Val615). The activation cleavage site is indicated by *arrowhead*. Matriptase is processed post-translationally via cleavage with an unknown intracellular processing enzyme after Gly149 within the SEA domain and that the C-terminal part (Ser150–Val855) is associated with cell membranes via anchoring with the N-terminal part (Met1–Gly149). The association is illustrated with *double broken lines*. Upon boiling in the presence of SDS, the N-terminal and C-terminal parts dissociate. The C-terminal part was visualized

at the position corresponding to 90 kDa upon SDS–PAGE under reducing conditions and Western blotting using Spr992. The molecular size of the catalytic domain (28 kDa) is indicated in the *lower illustration*. TM, transmembrane domain; SEA, sea-urchin sperm protein–enterokinase–agrin domain; C1 and C2, the first and second complement factor 1R–urchin embryonic growth factor–bone morphogenetic protein domains; L1–4, four LDLRA repeats; CD, catalytic domain. **b** Occurrence of WT-matriptase in the medium conditioned by the transfected COS-1 cells. COS-1 cells were transfected with pcDNA3.1(+) and pSecTag2/HygroB (Vector control), pcDNA-WT-matriptase and pSecTag2/HygroB (Matriptase alone), and pcDNA-WT-matriptase and pSec-HAI66K (Matriptase + HAI66K). Medium samples were separated by SDS–PAGE under reducing conditions; Western blot was probed with Spr992. The position at which the catalytic domain generated from activated protease migrates is indicated on the *right* by a *closed arrowhead*. Proteins with masses of approx. 90 kDa (indicated by an *open arrowhead*) and 45 kDa visible in the blot are non-specific bands representing unknown proteins. The molecular-mass standards are indicated on the *left* of blot in kDa

critical for the occurrence of matriptase in the extracellular environment.

Materials and methods

Anti-rat matriptase catalytic domain antibody

The procedure for the production of a rabbit polyclonal anti-matriptase antibody that recognizes a site within the catalytic domain (Ser682–Arg696, see Fig. 2a) (Spr992) was described previously (Tsuzuki et al. 2005).

Expression constructs

A plasmid for expression of a rat HAI-1 variant harboring the amino acids Pro41 to Leu513 (designated pSec-HAI66K) has already been constructed using pSecTag2/hygroB vector (Invitrogen, Carlsbad, CA, USA) (Tsuzuki et al. 2005). Plasmids for secreted variants of r-HAI-1 have also been constructed using the vector (Kojima et al. 2008). A plasmid for expression of WT-matriptase (designated pcDNA-WT-matriptase) has been constructed using pcDNA3.1(+) vector (Invitrogen) (Tsuzuki et al. 2005).

Transfection of expression plasmids into COS-1 cells and sample preparation

COS-1 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum as described previously (Tsuzuki et al. 2005). The trypsinized cells were plated in plastic 6-well plates (Asahi Techno Glass, Tokyo, Japan) for transient-expression experiments. The procedure for the transfection of constructs into the cells using Lipofectamine2000TM (Invitrogen) has been described previously (Tsuzuki et al. 2005). Cells were left undisturbed for 24 h after transfection. The transfected cells were then washed three times with phosphate-buffered saline (PBS) [8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 136 mM NaCl and 2.7 mM KCl (pH 7.4)], and then cultured for an additional 24 h in 1 mL of serum-free medium. Cells transfected with pcDNA-WT-matriptase alone were also incubated with serum-free medium containing 100 nM aprotinin (Takara Bio, Ohtsu, Japan) or serum-free medium conditioned by cells transfected with pSec-HAI66K alone. After incubation, the conditioned medium was harvested and transferred to a 1.5 mL microcentrifuge tube in which 100 µL of a protease inhibitor cocktail CompleteTM (10× concentrate) (Roche Diagnostics, Mannheim, Germany) was included. After centrifugation at 3,000g for 5 min at 22 °C, the resulting supernatant was concentrated to 40 µL by ultrafiltration using Microcon[®]-10 (10,000 NMWL, Millipore, Bedford, MA, USA). After addition of 10 µL of 5× Laemmli protein sample buffer (Laemmli buffer) [1× Laemmli buffer, 0.05 M Tris-HCl (pH6.8), 10% glycerol, 2% sodium dodecylsulfate (SDS) and 0.005% Bromophenol Blue with dithiothreitol at a final concentration of 12 mM] (Laemmli 1970), the ultrafiltrate was stored at −20 °C until use.

Analysis of expression of WT-matriptase and HAI-1 variants by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting

Samples were thawed, heated to 95 °C for 3 min, and subjected to SDS–PAGE (12% polyacrylamide). A 25-µL portion of samples was loaded onto each lane. After separation, the proteins were transferred by electroblotting onto a polyvinylidene fluoride

membrane (Fluorotrans W; Nihon Genetics, Tokyo, Japan) and then the blots were rinsed twice with a buffer (50 mM Tris-HCl, pH 7.5, containing 145 mM NaCl and 0.1% Tween 20) (hereinafter called TBST). The blots were being blocked by incubation with TBST containing 2% DifcoTM skim milk (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for 18 h at 4 °C. The signal for WT-matriptase was visualized as follows: after rinsing with TBST, the blots were incubated with Spr992 diluted in an immunoreaction-enhancer solution (Can Get Signal[®] Solution I, Toyobo) (1:20 dilution) for 18 h at 22 °C. After washing with TBST, the blots were incubated with a goat anti-rabbit IgG secondary antibody conjugated with HRP (Dako Japan, Kyoto) diluted in another immunoreaction-enhancer solution (Can Get Signal[®] Solution II, Toyobo) (1:3,000 dilution) for 2 h at 22 °C. After washing of blots with TBST, protein bands were visualized using ECL[®] detection system (GE healthcare, Tokyo branch). The HAI-1 variants were probed using horseradish peroxidase (HRP)-conjugated S-protein (Novagen, Madison, WI, USA) diluted in Can Get Signal[®] Solution II (1:5,000 dilution).

Results

Designation of r-HAI-1 variants

The domain structures of r-HAI-1 variants used in this study are illustrated in Fig. 1. An r-HAI-1 variant harboring the amino acids Pro41 to Leu513, including those for the transmembrane and cytoplasmic domains, was designated as HAI66K. For convenient detection, S-tag was fused to the N-terminus of the protein (Tsuzuki et al. 2005). This variant was shed from cell-surface membrane and was detected mainly as a 58-kDa band from medium samples (Miyake et al. 2009). We have recently shown that HAI66K inhibits secreted variants of r-matriptase *in vitro* (Kojima et al. 2009b).

A secreted variant of r-HAI-1 consisting of the entire extracellular domain (Pro⁴¹ to Leu⁴⁴¹) was designated as HAI58K, because it corresponds to an HAI-1 species with a molecular mass of 58 kDa that is known to occur via ectodomain shedding in cellulose (Shimomura et al. 1999). We have produced r-HAI-1 mutants by site-directed mutagenesis in the context of HAI58K, which we designate HAI58K-R260A and

HAI58K-K385A (Kojima et al. 2008). HAI58K-R260A and HAI58K-K385A are mutants in which Arg260 (in Kunitz domain I) and Lys385 (in Kunitz domain II) are replaced with Ala. These residues are considered essential for the potential inhibitory activities of the respective Kunitz domains (Denda et al. 2002). Secreted variants consisting only of Kunitz domain I (Gln245 to Ser307) and only of Kunitz domain II (Ser370 to Ser441) (Kojima et al. 2008) were designated as HAI-KD1 and HAI-KD2, respectively. All the secreted variants of r-HAI-1 were produced as fusion proteins with an S-tag at their N termini and a *Myc* epitope and hexahistidine tag at their C termini. We have shown that HAI58K, HAI58-K385A, and HAI-KD1 inhibit a secreted variant of r-matriptase *in vitro*, whereas HAI58K-R260A and HAI-KD2 do not (Kojima et al. 2008). The lack of inhibition activity of HAI58K-R260A and HAI-KD2 is not due to their misfolding. Indeed, the r-HAI-1 variants inhibited trypsin (Kojima et al. 2008).

Detection of WT-matriptase

WT-matriptase was detected rarely when expressed alone in COS-1 cells, but co-expression of HAI66K allowed the detection (Tsuzuki et al. 2005). Importantly, WT-matriptase was detected abundantly from the conditioned medium but poorly from a cell membrane-enriched fraction in the co-expression (Tsuzuki et al. 2005, Miyake et al. 2009). These findings suggested that most of WT-matriptase molecules are shed immediately after reaching the cell surface when expressed in this cell line. Therefore, the detection from medium samples is the convenient procedure for evaluating the extracellular occurrence of WT-matriptase.

The signals for non-activated and activated forms of WT-matriptase have been visualized at the position corresponding to 90 and 28 kDa, respectively, in SDS-PAGE under reducing conditions followed by Western blotting using an anti-matriptase catalytic domain antibody named Spr992 (refer to Fig. 2a), (Tsuzuki et al. 2005; Miyake et al. 2009). The 28-kDa band has been shown to represent the catalytic domain part of activated WT-matriptase (Satomi et al. 2001; Tsuzuki et al. 2005; Kojima et al. 2008, 2009a) (refer to Fig. 2a). Figure 2b shows that the medium sample from cells transfected with pcDNA-WT-matriptase and pSec-HAI66K produced a band at the 28-kDa

position (right lane, indicated by closed arrowhead), whereas those from cells transfected with parental vectors and pcDNA-WT-matriptase alone did not (left and middle lanes, respectively). These results confirm our previous findings (Tsuzuki et al. 2005; Miyake et al. 2009) that co-expression of HAI-1 is important for the detection of WT-matriptase from conditioned media.

In the present study, an immunoreaction-enhancer solution (Can Get Signal[®]) was used to sensitively detect WT-matriptase. Unexpectedly, the use of this solution resulted in the production of dense signals for unknown proteins around the position corresponding to 90 kDa (refer to Fig. 2b, left lane, indicated by open arrowhead). Therefore, the appearance of non-activated WT-matriptase in the medium samples could not be correctly evaluated. Nevertheless, the use of this solution indeed allowed better visualization of 28-kDa band. In the present study, the appearance of WT-matriptase in the conditioned media was evaluated by that of the 28-kDa band in blots. Hereinafter, the 28-kDa protein is referred to as simply WT-matriptase.

Importance of intracellular HAI-1 for the extracellular appearance of WT-matriptase

The lack of detection of WT-matriptase in the medium conditioned by cells transfected with pcDNA-WT-matriptase alone (refer to Fig. 2, middle lane) is thought to be due to its impaired intracellular trafficking or, for instance, to the intracellular self-degradation (Oberst et al. 2005). However, there remains the possibility that WT-matriptase occurs in the medium after which it undergoes self-degradation in the absence of HAI-1. To test the possibility of the extracellular self-degradation, COS-1 cells transfected with pcDNA-WT-matriptase alone were cultured with medium conditioned by those transfected with pSec-HAI66K alone. The extracellular self-degradation would be indicated if WT-matriptase was detected under the conditions employed. However, no signal for WT-matriptase molecules was detected (Fig. 3, lane 2). HAI66K was shown to be shed into the culture medium and was detected as a 58-kDa protein (Tsuzuki et al. 2005). The existence of HAI66K was confirmed in the medium samples (Fig. 3, lanes 4 and 5).

Aprotinin (a Kunitz-type protease inhibitor from ruminants) is known as a potent inhibitor of

matriptase (Yamasaki et al. 2003). In an in vitro assay, aprotinin inhibited HL-matriptase (Kojima et al. 2008) with an IC_{50} value of 3 nM (our unpublished work). WT-matriptase was not detected when cells transfected with pcDNA-WT-matriptase alone were cultured with serum-free medium containing 100 nM aprotinin (Fig. 3, lane 3). Results shown in Fig. 3 indicate that there was very little or no occurrence of WT-matriptase in the conditioned medium when expressed alone in COS-1 cells. In other words, these findings suggest the importance of intracellular HAI66K for the extracellular occurrence of WT-matriptase.

Requirement of the activity of HAI-1 for the extracellular appearance of WT-matriptase

WT-matriptase was co-expressed with secreted variants of r-HAI-1 with and without matriptase-inhibitory

activity in COS-1 cells, and the appearance of the protease in the conditioned medium was determined. Co-expressions of HAI58K and HAI58K-K385A allowed it, whereas that of HAI58K-R260A did not (Fig. 4). The appearance of r-HAI-1 variants in the media was examined by Western blotting using HRP-conjugated S-protein as the probe. HAI58K, HAI58K-K385A, and HAI58K-R260A were detected as a 59-kDa protein (Fig. 4). Note that the level of HAI58K-R260A was lower than those of HAI58K and HAI58K-K385A (Fig. 4).

Co-expression of HAI-KD1 allowed the appearance of WT-matriptase, whereas that of HAI-KD2 did not (Fig. 5). HAI-KD1 was visualized as a 15-kDa band (Fig. 5). However, no bands for HAI-KD2 were produced (Fig. 5). Results shown in Figs. 4 and 5 strongly suggest that the inhibition activity of HAI-1 toward matriptase is critical for the extracellular occurrence of WT-matriptase.

Fig. 3 Importance of intracellular HAI-1 for the extracellular occurrence of WT-matriptase in COS-1 cells. COS-1 cells were transfected with pcDNA-WT-matriptase and pSec-HAI66K (lanes 1 and 4) and with pcDNA-WT-matriptase alone (lanes 2, 3, 5, and 6). Twenty-four hours post transfection, cells were washed with PBS, exposed to fresh serum-free medium (lanes 1 and 4), serum-free medium conditioned for 24 h by cells transfected with pSec-HAI66K alone (lanes 2 and 5), and fresh serum-free medium containing 100 nM aprotinin (lanes 3 and 6), and incubated for 24 h. After incubation, media were harvested and concentrated by ultrafiltration. The medium samples were separated by SDS-PAGE under reducing conditions; Western blot was probed with Spr992 or HRP-conjugated S-protein (S-protein). The molecular-mass standards are indicated on the left of blot in kDa. The position at which the catalytic domain part of activated matriptase migrates is indicated on the left by an arrowhead

Fig. 4 Co-expression with HAI58K and its site-directed mutants. pcDNA-WT-matriptase was co-transfected with plasmids for expression of HAI58K, HAI58K-R260A, and HAI58K-K385A. The medium samples were subjected to SDS-PAGE under reducing conditions. After electronic transfer, the blot was probed with Spr992 or HRP-conjugated S-protein (S-protein). The molecular-mass standards are indicated on the left in kDa. The position at which the catalytic domain part of activated matriptase migrates is indicated on the left by an arrowhead

Fig. 5 Co-expression with HAI-KD1 and HAI-KD2. pcDNA-WT-matriptase was co-transfected with plasmids for expression of HAI-KD1 and HAI-KD2. The medium samples were subjected to SDS–PAGE (12% polyacrylamide) under reducing conditions. After electronic transfer, the blot was probed with Spr992 or HRP-conjugated S-protein (S-protein). For comparison, data from the sample of HAI58K-co-expressing cells is also indicated. The molecular-mass standards are indicated on the *left* of each blot in kDa. The position at which the catalytic domain part of activated matriptase migrates is indicated on the *left* by an *arrowhead*

Discussion

It is still unknown why co-expression of HAI-1 is important for the extracellular occurrence of matriptase in expression systems using BT549 and COS-1 cells. In the present study, we only presented evidence for the importance of intracellular HAI-1 (Fig. 3). An *in vitro* study using homogenates of a human mammary epithelial line 184 A1N4 (expressing both matriptase and HAI-1 endogenously) showed that matriptase underwent activation under acidic to neutral pH conditions (pH 5.2–7.0) and that the activation at pH 6.0 was strongly inhibited by fairly high ionic strength (140 mM NaCl) (Lee et al. 2007). This led to the suggestion that a number of matriptase molecules undergo activation inadvertently during their intracellular trafficking even in the presence of HAI-1 (Paroutis et al. 2004). We found that HL-matriptase was inhibited by HAI58K in buffers at pH and sodium ion concentration of intracellular environments (e.g., 100 mM MES, pH 5.5, containing

5 mM NaCl) (our unpublished observation). The most likely explanation for the effect of co-expression of HAI-1 is that HAI-1 inhibits matriptase activated inadvertently during intracellular trafficking to prevent the sequential self-degradation of the protease in COS-1 cells.

In the system using BT549 cells, matriptase was poorly produced when co-transfected with a cDNA for full-length r-HAI-1 bearing mutation at the active site Arg260 (designated R260L HAI-1) (Oberst et al. 2005). This suggests that the inhibition activity of HAI-1 toward matriptase is essential for production of this protease in the cell line. However, it has remained unknown whether R260L HAI-1 completely lacks matriptase-inhibitory activity. In the present study, we clearly showed that r-HAI-1 variants having matriptase-inhibitory activity allowed the occurrence of WT-matriptase in the conditioned media of COS-1 cells, whereas those completely lacking the activity did not. These results suggest that the matriptase-inhibitory activity of HAI-1, which is attributed to Kunitz domain I (Kojima et al. 2008), is critical for the extracellular occurrence of this protease. It is notable that co-expression of HAI58K gives comparable results to that of HAI66K. This suggests that cell-membrane anchoring is not essential for the matriptase-inhibitory activity of HAI-1 when expressed in COS-1 cells.

The protease-inhibitory activity of Kunitz domain II does not appear to affect the extracellular occurrence of WT-matriptase. Indeed, HAI58K and HAI58K-K385A gave comparable results (Fig. 4). In the present study, we found that HAI58K-R260A was detected at a low level in the co-expression with WT-matriptase (Fig. 4), this being consistent with the observation that R260L HAI-1 was produced poorly in the co-expression with matriptase (Oberst et al. 2005). It is plausible that the HAI58K-R260A variant, which lacks matriptase-inhibitory activity (Kojima et al. 2008), is susceptible to degradation by the protease. This idea is supported by the observation that HAI-KD2 did not occur in the medium samples. The poor detection of r-HAI-1 variants without matriptase-inhibitory activity also supports the idea that considerable active matriptase molecules could occur in the intracellular environment of COS-1 cells.

In the co-expression of full-length r-HAI-1 bearing mutation at Asp349 located in the low-density

lipoprotein receptor class A (LDLRA) domain (designated D349Y HAI-1), the activated matriptase occurred rarely (Oberst et al. 2003b). Therefore, the LDLRA domain of HAI-1 was postulated to play an important role in the process of matriptase activation (Oberst et al. 2003b). In the present study, we found that the level of activated WT-matriptase that occurred in the conditioned medium from HAI58K-co-expressing cells was similar to that from HAI-KD1-co-expressing cells (Fig. 5), suggesting that HAI-1 LDLRA domain per se has no significant effect on the activation of matriptase. One possible explanation for the effect of mutation at Asp349 is that this mutation in the context of full-length HAI-1 causes a conformational change that allows the inhibitor to interact with matriptase precursor in a cellular environment (i.e., on the cell surface). If so, the activation of matriptase via interaction between the precursors might be largely suppressed.

In summary, we showed that the inhibition activity of HAI-1 toward matriptase is critical for the extracellular occurrence of WT-matriptase in COS-1 cells. HAI-1 might prevent the self-degradation of matriptase in the intracellular environment. It has been shown that matriptase is not co-expressed with HAI-1 in certain cell types and cultured cells (e.g., in ovary granulosa cells and in human monocyte THP-1 cells) (Szabo et al. 2008; Kilpatrick et al. 2006). In addition, genetic studies using zebrafish indicated that matriptase does not require HAI-1 for biological activity (i.e., for extracellular occurrence) (Carney et al. 2007; Mathias et al. 2007). When HAI-1 is not co-expressed, HAI-2 (also known as bikunin) and the other serine protease inhibitors could prevent intracellular self-degradation of matriptase (Szabo et al. 2008). Regardless, the dual function of HAI-1 (inactivation of matriptase in the extracellular environment and the prevention of self-degradation in the intracellular environment) might be a mechanism allowing matriptase to be active temporarily at the site of action at least in cells expressing both the protease and inhibitor.

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References

- Bugge TH, List K, Szabo R (2007) Matriptase-dependent cell surface proteolysis in epithelial development and pathogenesis. *Front Biosci* 12:5060–5070
- Carney TJ, von der hardt S, Sonntag C et al (2007) Inactivation of serine protease Matriptase1a by its inhibitor Hail is required for epithelial integrity of the zebrafish epidermis. *Development* 134:3461–3471
- Darragh MR, Bhatt AS, Craik CS (2008) MT-SP1 proteolysis and regulation of cell-microenvironment interactions. *Front Biosci* 13:528–539
- Denda K, Shimomura T, Kawaguchi T et al (2002) Functional characterization of Kunitz domains in hepatocyte growth factor activator inhibitor type 1. *J Biol Chem* 277:14053–14059
- Désilets A, Béliveau F, Vandal G et al (2008) Mutation of G827R in matriptase causing autosomal recessive ichthyosis with hypotrichosis yields an inactive protease. *J Biol Chem* 283:10535–10542
- Kataoka H, Suganuma T, Shimomura T et al (1999) Distribution of hepatocyte growth factor activator inhibitor type-1 (HAI-1) in human tissues: cellular surface localization of HAI-1 in simple columnar epithelium and its modulated expression in injured and regenerative tissues. *J Histochem Cytochem* 47:673–682
- Kilpatrick LM, Harris RL, Owen KA et al (2006) Initiation of plasminogen activation on the surface of monocytes expressing the type II transmembrane serine protease matriptase. *Blood* 108:2616–2623
- Kim MG, Chen C, Lyu MS et al (1999) Cloning and chromosomal mapping of a gene isolated from thymic stromal cells encoding a new mouse type II membrane serine protease, epithin, containing four LDL receptor modules and two CUB domains. *Immunogenetics* 49:420–428
- Kim C, Cho Y, Kang CH et al (2005) Filamin is essential for shedding of the transmembrane serine protease, epithin. *EMBO Rep* 6:1045–1051
- Kojima K, Tsuzuki S, Fushiki T et al (2008) Roles of functional and structural domains of hepatocyte growth factor activator inhibitor type 1 in the inhibition of matriptase. *J Biol Chem* 283:2478–2487
- Kojima K, Tsuzuki S, Fushiki T et al (2009a) The activity of a type II transmembrane serine protease, matriptase, is dependent solely on the catalytic domain. *Biosci Biotechnol Biochem* 73:454–456
- Kojima K, Tsuzuki S, Fushiki T et al. (2009b) Role of the stem domain of matriptase in the interaction with its physiological inhibitor, hepatocyte growth factor activator inhibitor type I. *J Biochem* 145:783–790
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685
- Lee S, Dickson RB, Lin CY (2000) Activation of hepatocyte growth factor and urokinase/plasminogen activator by matriptase, an epithelial membrane serine protease. *J Biol Chem* 275:36720–36725
- Lee MS, Tseng IS, Wang Y et al (2007) Autoactivation of matriptase in vitro: requirement for biomembrane and

- LDL receptor domain. *Am J Physiol Cell Physiol* 293: C95–C105
- Lin CY, Tseng IC, Chou FP et al (2008) Zymogen activation, inhibition, and ectodomain shedding of matriptase. *Front Biosci* 13:621–635
- Mathias JR, Dodd ME, Walters KB et al (2007) Live imaging of chronic inflammation caused by mutation of zebrafish Hai-1. *J Cell Sci* 120:3372–3383
- Miyake Y, Yasumoto M, Tsuzuki S et al. (2009) Activation of a membrane-bound serine protease matriptase on the cell surface. *J Biochem* doi:10.1093/jb/mvp066
- Miyazawa K, Shimomura T, Kitamura A et al (1993) Molecular cloning and sequence analysis of the cDNA for a human serine protease responsible for activation of hepatocyte growth factor. Structural similarity of the protease precursor to blood coagulation factor XII. *J Biol Chem* 268:10024–10028
- Oberst MD, Singh B, Ozdemirli M et al (2003a) Characterization of matriptase expression in normal human tissues. *J Histochem Cytochem* 51:1017–1025
- Oberst MD, Williams CA, Dickson RB et al (2003b) The activation of matriptase requires its noncatalytic domains, serine protease domain, and its cognate inhibitor. *J Biol Chem* 278:26773–26779
- Oberst MD, Chen LY, Kiyomiya K et al (2005) HAI-1 regulates activation and expression of matriptase, a membrane-bound serine protease. *Am J Physiol Cell Physiol* 289:C462–C470
- Paroutis P, Touret N, Grinstein S (2004) The pH of the secretory pathway: measurement, determinants, and regulation. *Physiology* 19:207–215
- Satomi S, Yamasaki Y, Tsuzuki S et al (2001) A role for membrane-type serine protease (MT-SP1) in intestinal epithelial turnover. *Biochem Biophys Res Commun* 287:995–1002
- Shimomura T, Denda K, Kitamura A et al (1997) Hepatocyte growth factor activator inhibitor, a novel Kunitz-type serine protease inhibitor. *J Biol Chem* 272:6370–6376
- Shimomura T, Denda K, Kawaguchi T et al (1999) Multiple sites of proteolytic cleavage to release soluble forms of hepatocyte growth factor activator inhibitor type 1 from a transmembrane form. *J Biochem* 126:821–828
- Szabo R, Hobson JP, List K et al (2008) Potent inhibition and global co-localization implicate the transmembrane Kunitz-type serine protease inhibitor hepatocyte growth factor activator inhibitor-2 in the regulation of epithelial matriptase activity. *J Biol Chem* 283:29495–29504
- Takeuchi T, Shuman MA, Craik CS (1999) Reverse biochemistry: Use of macromolecular protease inhibitors to dissect complex biological processes and identify a membrane-type serine protease in epithelial cancer and normal tissue. *Proc Natl Acad Sci USA* 96:11054–11061
- Tsuzuki S, Murai N, Miyake Y et al (2005) Evidence for the occurrence of membrane-type serine protease 1/matriptase on the basolateral sides of enterocytes. *Biochem J* 388: 679–687
- Yamasaki Y, Satomi S, Murai N et al (2003) Inhibition of membrane-type serine protease 1/matriptase by natural and synthetic protease inhibitors. *J Nutr Sci Vitaminol (Tokyo)* 49:27–32
- Zhang T, Cai X, Schlegelberger B et al (1998) Assignment1 of human putative tumor suppressor genes ST13 (alias SNC6) and ST14 (alias SNC19) to human chromosome bands 22q13 and 11q24→q25 by in situ hybridization. *Cytogenet Cell Genet* 83:56–57